Bull. Environm. Contam. Toxicol. 21, 225-229 (1979)

Effects of Dietary Hexachlorobenzene on Distribution of Some Trace Metals in Rat Tissues

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Hexachlorobenzene (HCB) is registered for use as a fungicide in the treatment of common bunt in small grains. It is also produced as a by-product in the manufacture of organochlorine compounds and has been used in the production of pentachlorophenol and several other chemicals. Various reports (VOS et al. 1971, GILBERTSON and REYNOLDS 1972) have shown that the chemical may accumulate in the eggs of various wild birds. Recently, serious cases of HCB residues in cattle and sheep have been reported from Louisiana, Texas and California (BOOTH and MCDOWELL 1975). In addition, its toxicity to humans has been reported (SCHMID 1960) and its accumulation in human fat, milk, blood (BRADY and SIYALI 1972, ACKER and SCHULTE 1972, SIYALI 1972), and foods documented (SMYTH 1972).

The most frequent physiological responses to ingestion of the chemical by man and animals include porphyria and hepatomegaly. Hypertrichosis, weight loss, bone and joint lesions, nervousness and paralysis have also been observed in various species.

Because of a lack of information on the relationship between dietary HCB and the distribution of heavy metals in body tissues, the data reported here describe some findings on interactions between the chemical and certain metals.

MATERIALS AND METHODS

Twenty male, Sprague Dawley rats (279 ± 5 g) were allotted at random into two groups of 10 animals. Group 1 was the control, and group 2 was fed 250 µg of HCB/g diet (Purina Lab Chow[a]) over a period of 30 days. The control and treated animals were housed in separate rooms and had free access to food and water at all times. All animals were weighed both pre- and post-experiment.

After the experimental period of 30 days, all animals were killed, and liver, kidneys, spleen, and brain were collected and freeze dried for future analyses. All freeze dried organs and

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[a] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

0007-4861/79/0021-0225 $01.00

tissues were ground through a Wiley mill equipped with a 1.25 mm sieve. Digestion was by the nitric acid-perchloric acid method (A.O.A.C. 1970). Iron, copper, zinc, and manganese concentrations of all organs and tissues were determined by atomic absorption.

Analysis of variance and the least significance difference (LSD) were used to ascertain differences among treatments (STEEL and TORRIE 1960).

RESULTS

Both control and treated animals gained considerable weight during the experimental period. The greater increase in weight for the treated group (34%) than for the controls (29%) is probably the result of the increased (50%) weight of the liver and other organs. KIMBROUGH and LINDNER (1974) found a significant increase in weight of liver, lung, spleens, kidneys, and testes of rats fed high levels of HCB over a short period of time. No animals died during our experiment and treated animals showed no obvious signs of sickness. However, the dark-red urine of the treated animals may indicate excreted porphyrins.

Iron. The iron concentration of the liver and kidneys was significantly ($P < 0.05$) less in the HCB-treated animals than in the controls (Table 1). In addition, the iron concentration of the spleen and brains was markedly reduced by the HCB. Because blood hemoglobin and hematocrit levels were not determined, the anemic or non-anemic status of the animals could not be assessed. However, KIMBROUGH and LINDNER (1974) reported that animals fed 100, 500, and 1000 µg of HCB/g of diet became anemic within 4 months.

Copper. The liver copper concentration was significantly ($P < 0.05$) greater in the treated group than in the controls (Table 1). HCB had no significant effect on kidney, splenic, or brain copper concentrations.

Zinc. The average zinc concentration in the liver was significantly ($P < 0.05$) less in the treated animals than in the controls (Table 1). In contrast, the zinc concentration in the kidneys of the treated animals was significantly ($P < 0.05$) greater than in the controls (Table 1). The zinc concentration of the spleen and the brain were not significantly different between treatments.

Manganese. The manganese concentration of the liver, kidneys, and brain was significantly ($P < 0.05$) greater in the control animals than in treated animals (Tables 1 and 2). Furthermore, a marked depression was observed in the splenic manganese concentrations of the treated animals compared to the controls (Table 2).

TABLE 1

Average Concentration of Iron, Copper, Zinc and Manganese in Livers and Kidneys of Rats[a] Fed 250 µg/g of Dietary Hexachlorobenzene Over a Period of 30 Days.

Treatment	Fe		Cu		Zn		Mn	
	Liver	Kid.[b]	Liver	Kid.[b]	Liver	Kid.[b]	Liver	Kid.[b]
	µg/g Dry Tissue							
Control	770	560	13.9	25.0	89	91	7.40	3.63
Treated	610	460	15.7	23.7	81	101	5.93	3.07
S.E.	35	21	0.3	1.1	3	3	0.36	0.14
LSD (.05)	110	63	0.8	3.4	7	9	1.05	0.42

[a] 10 Control Rats and 10 Treated Rats
[b] Kid. = Kidney

TABLE 2

Average Concentration of Iron, Copper, Zinc and Manganese in Spleens and Brains of Rats[a] Fed 250 µg/g of Dietary Hexachlorobenzene Over a Period of 30 Days.

Treatment	Fe		Cu		Zn		Mn	
	Spl.[b]	Brain	Spl.[b]	Brain	Spl.[b]	Brain	Spl.[b]	Brain
	µg/g Dry Tissue							
Control	2445	109	7.26	20.9	113	57	8.08	1.91
Treated	1872	95	7.63	20.6	107	60	5.02	1.76
S.E.	241	8	0.42	0.4	5	1	1.15	0.04
LSD (.05)	716	23	1.26	1.3	14	3	3.42	0.13

[a] 10 Control Rats and 10 Treated Rats
[b] Spl. = Spleen

DISCUSSION

The data reported shows that 250 µg of HCB/g diet significantly depleted iron stores in liver and kidney and markedly reduced stores in spleen and brains of rats in a relatively short period of time. In contrast, SAUNDERS et al. (1963) reported that the feeding of HCB to rats resulted in the accumulation of iron in the liver and JOUBERT et al. (1973) confirmed these results. However, TALJAARD et al. (1972) reported that non-siderotic rats fed high concentrations of HCB for many months did not accumulate stainable iron to any significant degree. GOLDSTEIN (1976) also

reported that female rats fed high concentrations of HCB for 4 months did not accumulate significantly more liver iron than did control rats. No explanation can be given for the conflicting results, but some suggestions are in order. An effect by HCB on the intestinal absorption of iron seems reasonable because body iron stores were depleted. An indirect effect of HCB on red blood cell hemolysis due to the possible accumulation of porphyrins in the bone marrow (WATSON et al. 1959) also seems possible. This effect would cause the HCB-induced anemia reported by KIMBROUGH and LINDNER (1974) and may also cause the accumulation of iron in the liver, reported by the same authors, due to recycling of the iron content of the red blood cells. Whatever the mechanism, depletion and accumulation of iron in liver may both be part of HCB toxicity.

The significance of the decreased levels of zinc in the liver and the increased levels in kidneys due to HCB is not readily apparent. However, we do know that all forms of hepatic porphyrias are characterized by the urinary excretion of uroporphyrin and coproporphyrin I and III as zinc complexes. If we assume a limited accumulation of porphyrins in the livers of these rats, this accumulation may account for the decreased zinc in the livers.

The decreased levels of manganese in livers and kidneys due to HCB is consistent with that observed in cadmium poisoning of rats. Cadmiosis also causes anemia and a general depletion of iron throughout the body. The possible role of glucocorticoids in the depression of tissue manganese levels is not known; however, these hormones depress the hepatic uptake of ^{56}Mn (HUGHES and COTZIAS 1961). In addition, relatively high levels of dietary HCB cause hypertrophy of the adrenal cortex (KIMBROUGH and LINDNER 1974).

The increased hepatic copper levels due to HCB are consistent with our present knowledge of copper metabolism during acute stress (BEISEL and PEKAREK 1972).

The data reported here indicate that HCB, when fed to rats over a short period of time, caused changes in the iron, copper, zinc, and manganese concentrations of many tissues. Whether these changes are the result of changes in absorption, hemolysis of the red blood cells, accumulation of HCB and porphyrins in the tissues or a combination of some or all of these factors in unknown. The supplementation or deprivation of some dietary essential metals may have some effect in counteracting the toxic effects of the fungicide. Further studies are needed to answer these questions.

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